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Kinetic Parameters for the Biological Treatment of Mixed Wastes Containing Acetonitrile and Methanol

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Executive Summary

This research was conducted to investigate the feasibility of applying microbial biodegradation as a treatment technology for mixed wastes. In this study, we focused our efforts on the treatment of wastes generated by biomedical research during the purification of tritium labeled compounds by high-performance liquid chromatography (HPLC). These wastes are typically 80% water with 20% acetonitrile or methanol or a mixture of both. The objective was to determine the potential of using biodegradation to treat the solvent component of tritiated mixed waste to a concentration below the land disposal restriction standard. Once the standard is reached, the remaining radioactive waste is no longer classified as a mixed waste and it can then be solidified and placed in a secure landfill. This approach would be superior to incineration, because radioactivity would not be released into the atmosphere during this process.

Bacterial culture were developed that could grow on methanol and acetonitrile at high concentrations. The growth and substrate oxidation kinetics for these cultures were measured in order to set boundary criteria for the design of a pilot scale biological treatment process. The degradation of the HPLC solvents, acetonitrile and methanol, followed well known kinetic models (Monod or Haldane), indicating that scale-up of the biodegradation process to pilot or full-scale treatment can follow standard engineering protocols. Half-saturation constants, inhibition constants, maximum oxidation rates and maximum growth rates were determined for each solvent degrading bacterial culture. Acetonitrile was found to be more difficult to degrade than methanol. Acetonitrile was an inhibitory substrate and had a higher half-saturation constant than methanol. The results indicate that the biological treatment plant design should primarily be based on the criteria necessary for the destruction of acetonitrile.

These studies indicate that it is technically feasible to use bacterial oxidation to treat mixed wastes. Mixed wastes containing methanol and acetonitrile as their main organic components can be treated to meet land disposal restriction standards of less than 1 mg/L. The results of this study will be used to design bench scale reactors for the treatment of tritiated mixed wastes.

Introduction

Tritiated organic compounds with high radiochemical purity are important tools for biomedical research and are widely used in the United States and internationally. The National Tritium Labeling Facility (NTLF) at Lawrence Berkeley National Laboratory (LBNL) has been an important manufacturer of tritium labeled compounds used in biomedical research. As part of the tritium labeling process, the labeled compounds are purified by high-performance liquid chromatography (HPLC). The HPLC purification process most commonly uses acetonitrile and methanol as solvents. After the product is isolated, the spent solvent mixture may contain acetonitrile, methanol, water, and minor organic ingredients (such as trifluoroacetic acid), as well as tritium. Although incineration of this waste is permitted, it is not a desirable method because the tritium in the waste is released to the environment during incineration.

The HPLC waste stream is hazardous organic waste subject to Resource Conservation and Recovery Act (RCRA) regulations by the Environmental Protection Agency (EPA). In addition, the radioactive portion of the mixed waste is low level waste subject to regulation under the Atomic Energy Act by the Nuclear Regulatory Commission and Department of Energy .

Under RCRA, D-coded organic solvents must be treated to meet concentration-based or technology-specific land disposal restrictions [1] before they may be disposed (Table 1). The HPLC waste stream is designated as the D001 ignitable non-wastewater hazardous waste under RCRA. In particular, the D001 designation means that the HPLC waste must be treated by a defined technology standard. Accepted technology standards for acetonitrile and methanol are solvent recovery (RORGS), polymerization (POYLM) and combustion (CMBST) [2]. Two treatment standards (RORGS and POLYM) are not practical for the treatment of mixed wastes containing tritium. For the wastes generated by the NTLF, combustion is the only currently available, approved treatment. However, available offsite commercial options for performing the RCRA-required treatment (CMBST) are not desirable in LBNL's view. LBNL has adopted a zero discharge policy for tritium, and the release of tritium into the atmosphere during combustion, although safe and allowable, is not desirable. Additionally, incineration cost are expected to amount to several million dollars. Based both on environmental consequences and the expense, LBNL is interested in developing an alternative treatment strategy for mixed wastes that can efficiently destroy the organic compounds in the wastes without releasing tritium to the environment. This treatment technology could then be used for the treatment of biomedical mixed wastes generated at hospitals, universities, and research institutes.

HPLC Waste Composition and Treatment Standards

The spent solvents generated from the high performance liquid chromatography (HPLC) purification process may consist of the following compounds: water, acetonitrile, methanol, hexane, tetrahydrofuran, ethanol, isopropanol, trifluoroacetic acid, or chloroform (Table 2). These wastes typically contain 40–80% water and 20–40% organic content. Acetonitrile and methanol are D001 wastes that must meet a treatment standard (Table 1). In practical terms, this means that acetonitrile and methanol must be reduced to a final concentration of less than 1 mg/kg in the final solidified waste.

Study Purposes and Scopes

The purpose of this study was to determine if biological treatment could be an efficient and environmentally preferable alternative to incineration. We studied the degradation of the two major hazardous chemicals found in HPLC waste, acetonitrile and methanol. We developed mixed cultures capable of degrading acetonitrile and methanol at high concentrations. We measured bacterial growth and substrate degradation as a function of acetonitrile and methanol concentration. From these experiments we determined the biokinetics governing the treatment of these chemicals and developed recommendations for treatment process design.

Literature Review

Previous biodegradation studies [3-11] demonstrated that a variety of microorganisms could degrade organic nitriles including acetonitrile. The microbial metabolism of nitriles can proceed through either a single-step or two-step pathway. The single-step enzymatic nitrilase converts nitriles directly to carboxylic acid and ammonia. The two-step pathway involves a hydratase that converts nitriles to amides, and then an amidase that converts the amides to carboxylic acid and ammonia.

Nawaz *et al.* [3] studied a bacterium, *Pseudomonas putida*, that is capable of utilizing high concentrations of acetonitrile as the sole source of carbon and nitrogen. The bacteria produced acetic acid and ammonia as products of acetonitrile degradation. The acetate was formed in the early stages of growth and was consumed in the later stages of the fermentation. Cells grown on acetonitrile contained active nitrile hydratase and amidase. Both enzymes were intracellular, inducible, and hydrolyzed a wide range of substrates. The specific activity of amidase was at least 150-fold higher than the activity of the enzyme nitrile hydratase.

The same research laboratory also isolated a *Pseudomonas aeruginosa* capable of growing on acetonitrile as the sole source of carbon and nitrogen and biphenyl as a sole source of carbon [4]. This *Pseudomonas aeruginosa* metabolized acetonitrile to ammonia and acetic acid and biphenyl to benzoic acid. They also found that the bacterium can simultaneously utilize biphenyl as the sole carbon source and acetonitrile as the sole nitrogen source. Biphenyl utilization rate increased after the depletion of acetonitrile. Metabolites of the mixed substrate were ammonia and benzoic acid, which completely disappeared in the later stages of incubation.

Another acetonitrile degrading bacteria, *Rhodococcus erythropolis* BL1, was isolated from coastal marine sediments [5]. This organism can grow on acetonitrile as the sole carbon and nitrogen source. Intact cells of *R. erythropolis* BL1 could hydrolyze a large variety of saturated and unsaturated aliphatic nitriles to their corresponding acids. Detailed studies showed that nitrile hydrolysis by strain BL1 was due to a nitrile hydratase/amidase enzyme system. Nitrile hydratase activity was found to be inducible whereas amidase activity was constitutive. The amidase activity could, however, be enhanced many fold by growth in media containing acetamide or acetonitrile. In most cases amides were hydrolyzed at a much higher rate than the corresponding nitriles. *R. erythropolis* BL1 exhibited the high tolerance towards acetonitrile as demonstrated by its ability to grow exponentially in the presence of 0.9 M (i.e., 36,900 mg/liter or ~3.7%) acetonitrile.

A variant of a yeast strain, *Candida famata* isolated from a gold mine effluent by Linardi et al [6], was able to grow on acetonitrile, acrylonitrile, butyronitril, isobutyronitrile, methacrylnitrile, propionitrile, succinonitrile, valeronitrile, acetamide, isobutyamide, and succinamide as sole nitrogen source, after acclimatization. The yeast grew on acetonitrile and acetamide at concentrations up to 4%. However, best growth of the yeast was seen when 1% acetonitrile was used as the sole source of nitrogen. The utilization of acetonitrile and acetamide by the *C. famata* strain is believed to involve hydrolysis in a two-step reaction mediated by both inducible and intracellular nitrile hydratase and amidase.

The optimum conditions for the formation of a nitrile hydratase, which acts on indole-3-acetonitrile, was examined in *Agrobacterium tumefaciens* [7]. Inducers of enzyme formation were classified into three representative types of amides: pivalamide, crotonamide and c-caprolactam. When the strain was cultivated in the optimum culture medium containing c-

caprolactam as an inducer the specific activity of nitrile hydratase in the culture was 13,000 times higher than that without addition of amides, nitriles or acids. This suggests that c-caprolactam seems to keep driving the nitrile hydratase induction mechanism.

A petrochemical wastewater isolate, capable of utilizing high concentrations of acetonitrile and acetamide as the sole source of carbon and nitrogen was identified as *Rhodococcus erythropolis* A10 [8,9]. *Rhodococcus erythropolis* A10 used a two-step process involving nitrile hydratase and amidase for metabolizing acetonitrile. Studies indicated that both these enzymes in *R. erythropolis* A10 are intracellular, inducible and capable of hydrolyzing a wide range of nitriles, including simple (acetonitrile, propionitrile), branched-chain (isobutyronitrile) and dinitrile (succinonitrile). The specific activity of the amidase was found to be several-fold higher than nitrile hydratase. The strain was able to maintain activity at concentrations of up to 3% acetonitrile. Nitrile hydratase activity was dependent on cobalt and iron, while amidase showed a dependence on iron. The presence of glucose or ammonium sulfate did not affect acetonitrile utilization.

Two previous studies have been conducted examining the biological treatment of mixed wastes. Wolfram *et al.* [10] tested the biotreatability of mixed waste samples obtained from the National Institute of Health. The typical waste sample used for this study contained methanol, acetonitrile, and pseudocunene. The process demonstrated that a selected mixed population of microorganisms could successfully degrade a mixture of methanol and acetonitrile in 2 to 7 days, but if the methanol and acetonitrile were mixed with pseudocunene, the degradation was inhibited and was not complete until 27 days. A second study examined the biodegradation of liquid scintillation cocktail containing low-level tritium and plutonium [11]. In this study, pseudocunene was the primary organic constituent. The LSC was completely degraded in 2 days.

Materials and Methods

Chemicals

Acetonitrile; zinc sulfate, heptahydrate ($ZnSO_4$-$7H_20$); cupric sulfate, pentahydrate ($CuSO_4$-$5H_2O$); and potassium phosphate, monobasic (KH_2PO_4) were purchased from EM Science, Gibbstown, NJ. Sodium phosphate, dibasic, anhydrous (Na_2HPO_4); ammonium chloride (NH_4Cl); and sodium molybdate, dihydrate (Na_2MoO_4-$2H_20$) were purchased from JT Baker, Phillipsburg, NJ. Magnesium sulfate, heptahydrate ($MgSO_4$-$7H_2O$) and calcium chloride, dihydrate ($CaCl_2$-$2H_2O$), were purchased from Fisher, Fairlawn NJ. Hydrochloric acid (HCl) and ferrous sulfate, heptahydrate ($FeSO_4$-$7H_2O$) were purchased from Sigma Chemical Co., St. Louis, MO. Manganese sulfate, monohydrate ($MnSO_4$-H_2O) was purchased from Mallinckrodt Baker, Paris, KY.

Media Preparation

The minimal salts media used for the enrichment cultures and the growth curves contained the following: 2,000 mL distilled water (Millipore Quantity, > 18 Ohms); KH_2PO_4, 2.00 g; Na_2HPO_4, 1.72 g; NH_4Cl, 2.00 g; $MgSO_4$-$7H_2O$, 0.24 g; $CaCl_2$-$2H_2O$, 0.12g. Finally, 2 mL of a trace metals solution was added; it contained: 995.8 mL distilled water (Millipore Quantity, > 18 Ohms); HCl, 4.15 mL; $MnSO_4$-H_2O, 3.3 mg; $CuSO_4$-$5H_2O$, 6.2 mg; $ZnSO_4$-$7H_20$, 7.6 mg; Na_2MoO_4-$2H_20$, 11.7 mg; and $FeSO_4$-$7H_2O$, 64.6 mg. Media is autoclaved for twenty minutes.

Analysis of Acetonitrile and Methanol

Acetonitrile and methanol were analyzed by direct aqueous injection using a Hewlett Packard 6890 gas chromatograph (GC) equipped with a flame ionization detector (FID). Samples were collected from the cultures and either filtered or centrifuged to remove biomass. Samples were placed on an autosampler and 0.2 uL of sample was injected into the GC. Separation was achieved on a J&W DB-624 30.0 m by 250 um by 1.40 um capillary column, using helium as a carrier gas, a 6:1 split ratio, an inlet temperature of 225° C, and a column flow of 0.7 mL/min. The column oven was set to an initial condition of 40° C for 4 minutes followed by a 16° C per minute increase to 150°, held for 3 minutes, for a total run time of 13.9 minutes. The FID detector was maintained at 250° C with hydrogen at 40 mL/min, air at 450 mL/min, and nitrogen as a makeup gas at 45 mL/min. The lower detection limit for acetonitrile and methanol by this method is 0.2 mg/L.

Enrichment Culture

A sample of dry soil was obtained from an uncontaminated DOE site. Two grams of the soil was added to 50 mL of distilled water and well mixed. After the soil settled, one mL of the supernatant was added to 300 mL of the minimal salts media described above. One mL of acetonitrile or methanol was added as the sole carbon source. After the culture became turbid due to the bacterial growth, another 100 mL of the minimal salts media was added to the culture. After the culture began to grow, 200 mL of the enrichment was poured off and replaced with 200 mL of the minimal salts media and 0.5 mL of acetonitrile or methanol. This was routinely done twice a week to keep the culture active and healthy.

Growth Curve Assay

All glassware and instruments used were sterilized in the autoclave. Twenty mL of the acetonitrile or methanol enrichment was centrifuged and washed three times with minimal salts media. After washing, the culture was re-suspended in the minimal salts media to 20 mL. Sample solutions of acetonitrile or methanol in the minimal salts media were prepared at concentrations ranging form 40 mg/L to 100,000 mg/L. 50 mL of each concentration was added into it's own sidearm flask (path length = 13 mm). Four drops of the washed acetonitrile or methanol enrichment were added to each side arm flask and the flasks were tightly sealed with caps. The initial optical density of each flask was recorded and then the flasks were placed on an orbital shaker (250 rpm's at 25° C). Optical density measurements were taken approximately every half.

Five mL samples were drawn from each flask at the beginning of log phase and at the end of log phase. These samples were preserved with one drop of 85% phosphoric acid and placed in the refrigerator. When the flasks reached the stationary growth phase (about 6-7 days for acetonitrile or 4 to 5 days for methanol), the flasks were preserved with two drops of 85% phosphoric acid. The samples were analyzed for acetonitrile or methanol concentration on the Gas Chromatograph (GC).

Growth rates for each concentration of acetonitrile or methanol were obtained by graphing the natural log of the optical density as a function of time. The slope of the linear portion of the graph is the initial growth rate of the culture. Growth rate as a function of acetonitrile or methanol concentration was graphed and a Monod or Haldane model (see below for Equation [1] or [2], respectively) was fit to the data. The kinetic parameters K_s (half-saturation constant) and μ_{max} (maximum growth rate) were obtained from the model fit using nonlinear regression.

Respirometry Assay

Two vials of acetonitrile or methanol culture were centrifuged and washed three times in a minimal salts media. After the third washing, the culture was re-suspended to 30 mL in minimal salts media in each vial and the optical density was measured. The culture was placed into a water bath of the respirometer at 25° C.

The respirometer (Clark type electrode) was calibrated with distilled water saturated with oxygen. Approximately 1.7 mL of the acetonitrile or methanol culture was then placed into the respirometer. The oxygen uptake of the culture was monitored for about ten minutes, until a steady baseline slope was obtained. Substrates (solutions of acetonitrile or methanol in distilled water) were prepared, for example, ranging from 10 mg/L to 10,000 mg/L, when injected into the cell. 10 µL of the acetonitrile or methanol solution is injected into the cell. The oxygen uptake of the culture was monitored for another ten more minutes before the culture in the cell was removed. The cell was washed several times with distilled water and then fresh culture was added for the next test.

Oxygen uptake rates were calculated by graphing oxygen uptake as a function of time, calculating the slopes of the first portion of the graph (before the substrate injection) and the second portion of the graph (after the substrate injection), and taking the difference between them for each concentration. Then the oxygen uptake rate as a function of acetonitrile or methanol concentration was graphed and fit with the Monod or Haldane model. The kinetic

parameters K_s (half-saturation constant) and μ_{max} (the maximum growth rate) were determined by the fit.

Kinetic Models

Knowledge of the kinetics of biodegradation is important for the evaluation of the persistence of organic solvents and the design of a biodegradation process. Three kinetic models, Monod (Equation 1), Haldane (Equation 2), and Michaelis-Menten (Equation 3), were considered in this study. The Monod and Haldane models were applied to growth data. The Michaelis-Menten model was applied to the respirometry data. The models are as follows:

$$\mu = \frac{\mu_{max} * [S]}{K_s + [S]} \qquad [1]$$

$$\mu = \frac{\mu_{max} * [S]}{K_s + [S] + [S]^2/K_i} \qquad [2]$$

$$v = \frac{V_{max} * [S]}{K_s + [S]} \qquad [3]$$

in which μ is the specific growth rate, μ_{max} is the maximum specific growth rate, v is the specific oxidation rate, V_{max} is the maximum oxidation rate, K_s is the half-saturation constant for substrate utilization or cell growth, and K_i is the inhibition constant for cell growth on substrate.

Results and Discussion

Kinetics of Acetonitrile and Methanol Biodegradation

Cell growth rate as a function of acetonitrile concentration was examined for concentrations of acetonitrile up to 100,000 mg/L (Figure 1). Acetonitrile was found to be inhibitory to growth at high concentrations, although there was some growth even at 100,000 mg/L (10% acetonitrile in water). The data from this experiment was fit to a the Haldane model. The kinetic parameters for the Haldane model are reported in Figure 1.

In the operation of a pilot or full-scale treatment system it is generally preferred to avoid inhibitory concentrations of substrates. Cell growth rate as a function of acetonitrile concentration was again examined at a series of lower concentrations, with a maximum acetonitrile concentration of 5,000 mg/L (Figure 2). At this lower concentration range, bacterial growth on acetonitrile could be fit to the Monod model. The Monod model estimated the maximum growth rate (μ_{max}) on acetonitrile to be 5.2 day^{-1} with a K_s of 68 mg/L.

In the mixed wastes generated by a biomedical research laboratory, the methanol concentration rarely if ever exceeds the acetonitrile concentration; therefore we only tested methanol at the lower concentration in the treatment process. Cell growth rate as a function of methanol concentration was examined for concentrations of methanol up to 16,000 mg/L (Figure 3). Methanol was an excellent substrate for bacteria growth and growth was not inhibited even at the maximum concentration tested (Figure 3). The maximum growth rate on methanol was also approximately 5 day^{-1}. We did not estimate K_s for methanol using this technique, because we did not have a sufficient number of measurements at a low enough concentration range.

To get the most accurate measurements of K_s possible, we conducted respirometry experiments using a Clark-type electrode. We measured the acetonitrile oxidation rate as a function of concentration up to 10,000 mg/L without observing an inhibitory effect. The data from this experiment was fit to the Michaelis-Menten model to estimate K_s and V_{max} (Figure 4). The Ks estimated by this method was 89 mg/L, which is not significantly different from our estimate from the growth rate measurements (68 mg/L). Estimates of half-saturation constants (K_s) made by respirometry are typically more accurate than estimates made from growth tests, because respirometry is a more instantaneous measurement. (Growth rate is measured over days; respirometry is measured over minutes). We will use the respirometry estimates for design of the pilot scale treatment process.

The oxidation of methanol as a function of concentration was also investigated. A series of experiments demonstrated that the K_s for methanol was less than 2 mg/L (data not shown). A definitive experiment was then executed to determine a best estimate of K_s for this compound. Our best estimate for methanol is a half saturation constant of 0.16 mg/L (Figure 5).

The results of these experiments suggest that acetonitrile concentration will be a limiting parameter for the design of an aerobic biological treatment process. It is apparent that the reaction rate will decrease if the acetonitrile concentration is too high (e.g., > 1%). Additionally the K_s for acetonitrile is large, suggesting that the efficiency of the bacterial biodegradation system responsible for acetonitrile degradation is low. In contrast, methanol did not appear inhibitory at the concentrations tested. Additionally, the low K_s for methanol suggests that the treatment of methanol will remain efficient even at low residual methanol concentrations.

Treatment of Solvents to Concentrations Acceptable for Land Disposal

Samples of acetonitrile or methanol were periodically withdrawn from batch degradation experiments and analyzed by a GC. The GC analytical data (see Tables 3 and 4) of several tests demonstrated that both acetonitrile and methanol were biodegraded. When sufficient nutrients, substrates, and oxygen were supplied to the mixed bacteria culture, the concentrations of acetonitrile and methanol can be reduced to less than 1 mg/L. Our results also indicated that when the initial acetonitrile or methanol concentration in the flask was $\leq$ 1,000 mg/liter, the substrate was degraded to non-detected level within 10 days.

Effect of Trifluoroacetic Acid on Acetonitrile Degradation

We also examined the effects of a minor ingredient on the degradation performance of major solvents. Several respirometry tests and batch degradation tests were performed with surrogate solvent mixtures containing mainly acetonitrile (1,000 mg/liter) with trace trifluoroacetic acid (TFA); concentration ranges from 0.5 to 5 mg/liter).

Our preliminary results (Figure 6) indicate that TFA did not inhibit the degradation of acetonitrile. However, further studies are needed to better understand how TFA and other trace constituents (Table 2) influence the degradation of acetonitrile and methanol.

Conclusions

In this study, we developed mixed cultures capable of degrading the major solvent components (acetonitrile and methanol) of HPLC waste. Kinetic parameters governing the degradation of acetonitrile and methanol by the enriched mixed cultures were determined. The degradation of the HPLC solvents, acetonitrile and methanol, followed well known kinetic models, indicating that scale-up of the biodegradation process to pilot or full-scale treatment can follow standard engineering protocols. Half-saturation constants, inhibition constants, maximum oxidation rates and maximum growth rates were determined for each solvent degrading bacterial culture. Acetonitrile was found to be more difficult to degrade than methanol. Acetonitrile was an inhibitory substrate and had a higher half-saturation constant than methanol. The results indicate that biological treatment plant design should primarily be based on the criteria necessary for the destruction of acetonitrile.

Our test results indicated that biological degradation has potential to serve as an alternative treatment technology for HPLC mixed wastes. Under many conditions, residual solvent concentrations were reduced to less than 1.0 mg/L. Wastes with less than 1.0 mg/L residual solvent are not longer classified as mixed wastes and can be sent to a radioactive waste landfill. Preliminary tests indicate that the trace constituents in HPLC mixed wastes, such as TFA, will not interfere with the biodegradation process. These results indicate that a practical biotreatment technology can be developed to replace incineration for the treatment of this type of waste. The advantage of biological treatment is that no tritium would be released during the treatment of the organic component.

This research is continuing. Bench scale pilot reactors have been designed and are being tested for the treatment of surrogate mixed wastes. Future research will examine the impact of individual trace components on the biodegradation methanol and acetonitrile alone and in mixture. Reactor design will be finalized at the completion of these experiments and a mixed waste containing tritium will be treated to demonstrate complete containment and treatment.

Acknowledgement

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References

(1) USEPA, "Land Disposal Restrictions," 40 CFR 268.40; in *RCRA Regulations and Keyword Index*, Elsevier (1999).

(2) USEPA, "Treatment Standards Expressed as Specified Technologies," 40 CFR 268.42, Table 1; in *RCRA Regulations and Keyword Index*, Elsevier (1999); and USEPA, "Universal Treatment Standards," 40 CFR 268.48; in *RCRA Regulations and Keyword Index*, Elsevier (1999).

(3) Mohamed S. Nawaz, Kirit D. Chapatwala, and James H. Wolfram, 1989, Degradation of Acetonitrile by *Pseudomonas putida,* Appl. Environ. Microbiol., 55:2267 – 2274.

(1) Mohamed S. Nawaz, and Kirit D. Chapatwala, 1991, Simultaneous degradation of acetonitrile and biphenyl by *Pseudomonas aeruginosa*, Can. J. Microbiol., 37: 411 – 418.

(2) Bjarne R. Langdahl, Peter Bisp, and Kjeld Ingvorsen, 1996, Nitrile hydrolysis by *Rhodococcus erythropolis* BL1, an acetonitrile-tolerant strain isolated from a marine sediment, Microbiology 142: 145 – 154.

(3) Valter R. Linardi, Joao C.T. Dias, and Carlos A. Rosa, 1996, Utilization of acetonitrile and other aliphatic nitriles by a *Candida famata* strain, FEMS Microbiol. Letters 144: 67 –71.

(4) M. Kobayashi, T. Fujita, and S. Shimizu, 1996, Hyperinduction of nirtile hydratase acting on indole-3-acetonitrile in *Agrobacterium tumefaciens*, Appl. Mirobiol. Biotechnol., *45: 176 – 18.*

(5) A. Acharya, and A.J. Desai, 1997, Studies on utilization of acetonitrile by *Rhodococcus erythropolis* A10, World J. Microbiol. & Biotechnol., 13: 175 – 178.

(6) A. Acharya, and A.J. Desai, 1999, Short Communication: Acetonitrile-hydrolysing enzymes in *Rhodococcus erythropolis* A10, World J. Microbiol. & Biotechnol., 15: 123 – 125.

(7) J.H. Wolfram, M. Radtke, J.E. Wey, R.D. Rogers, and E.H. Rau "Degradation of Hazardous Chemicals in Liquid Radioactive Wastes from Biomedical Research Using Mixed Microbial Population", Proc. 4th ASME Mixed Waste Symposium, **1997**. published in *Technology: J. Franklin Institute* **334A**, 171–184 (1997).

(8) J.H. Wolfram, R.d Rogers, R. Finney, A. Attala, G. Silver, and F. Hertwieck, Jr. "Bioprocessing of Mixed Waste: A Success Story", Proc. 3rd Mixed Waste Symposium, **1995**.

Table 1. EPA Codes of HPLC Solvents and Universal Treatment Standards

EPA Code	HPLC Solvent Ingredients	Universal Treatment Standard (UTS) for Nonwastewater (high TOC)
D001	Acetone, ACN, EtOH, EtOAc, ether, MeOH, THF, IPA, DMF	CMBST, RORGS, or POLYM
D022	chloroform (> 6 ppm TCLP)	6 mg/kg

Note: CMBST = combustion, RORGS = recovery of organics, POLYM = polymerization, TOC = total organic compounds.

Table 2. Examples of HPLC Waste Composition

Water Content	Solvent Content
60 - 80%	ACN (20 - 40%) with trace of THF, TFA, MeOH, IPA, hexane, EtOAc,
60 – 80%	MeOH (20–40%), with trace of THF, TFA, ACN.
80%	ACN (10%) and MeOH (10%).
35 – 80%	ACN (20–65%), with trace of THF, MeOH, EtOH, IPA, TFA, DMF

Note: ACN = acetonitrile, DMF = dimethyl formamide, EtOAc = ethyl acetate, EtOH ethanol, IPA = isopropanol, MeOH = methanol, THF = tetrahydofuran, TFA = trifluoroacetic acid

Table 3. Acetonitrile (ACN) Degradation Tests Using the ACN-Enriched Mixed Culture

Initial ACN Concentration (mg/L)	ACN Concentration (mg/L) on Day 3	% Degradation after 3 days	ACN Concentration (mg/L) on Day 4	% Degradation after 4 days	ACN Concentration (mg/L) on Day 8	% Degradation after 8 days
40	22.2	44.9	0.0	100	0.0	100
100	62.5	37.5	0.0	100	0.0	100
500	356	28.8	0.0	100	0.0	100
700	530	24.3	163	76.8	0.0	100
1000	830	17.0	441	55.9	0.0	100
5000	4404	11.9	4044	19.1	1862	62.8

Table 4. Methanol (MeOH) Degradation Tests Using the MeOH-Enriched Mixed Culture

Initial MeOH Concentration (mg/L) on Day 0	Initial mg MeOH in each flask	MeOH Concentration (mg/L) on Day 2	% MeOH Degraded after 2 Days	MeOH Concentration (mg/L) on Day 6	% MeOH Degraded after 6 Days
48	2.40	0	100	0	100
106	5.29	0	100	0	100
548	27.4	430	21.4	0	100
746	37.3	596	20.1	0	100
1093	54.6	881	19.3	0	100
5308	265	4602	13.3	3453	34.9

Figure 1: Growth of bacteria on acetonitrile. High concentrations of acetonitrile are inhibitory to growth.

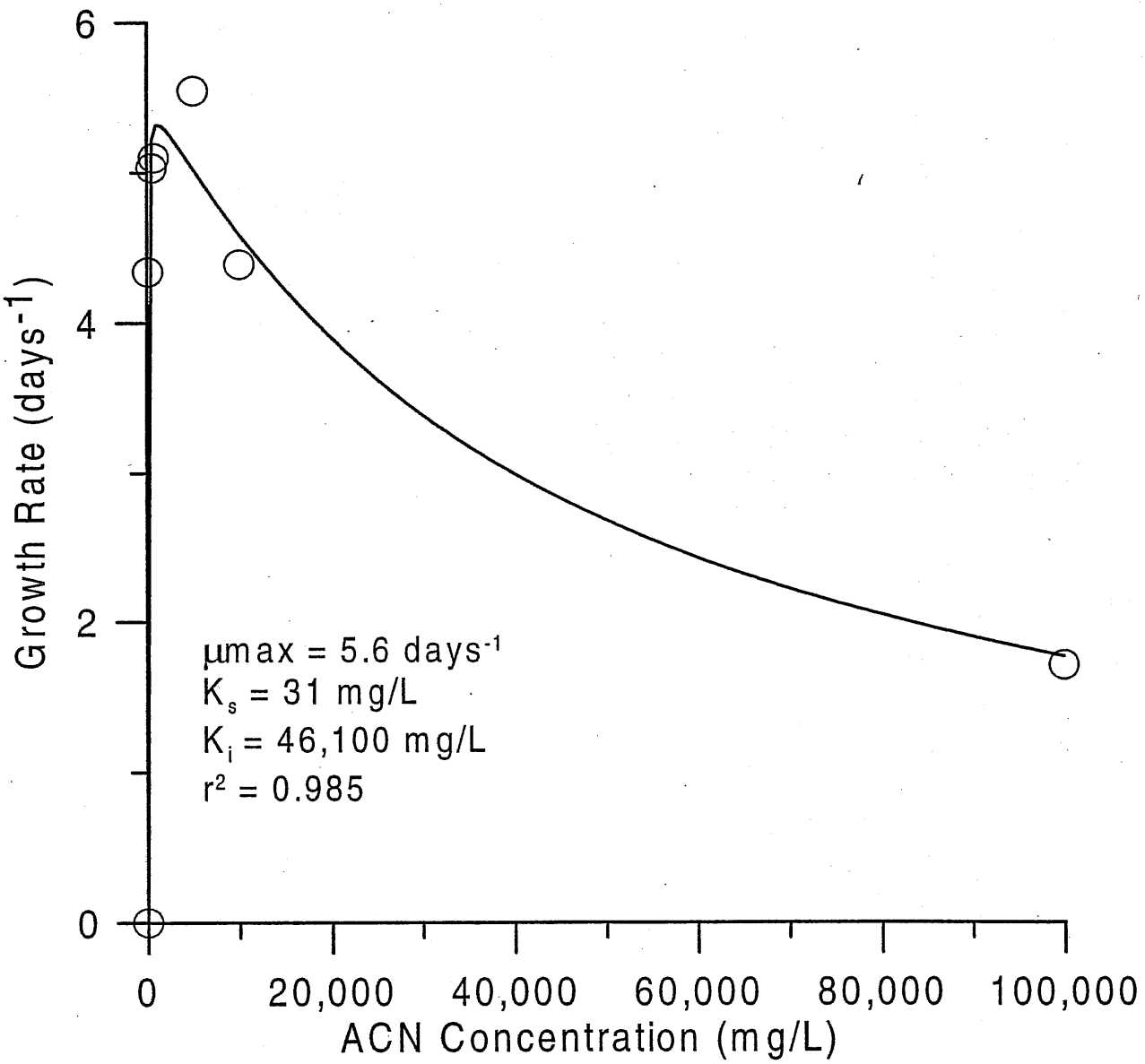

Figure 2: Growth of bacteria on acetonitrile. At lower acetonitrile concentrations, bacterial growth can be described using the Monod model

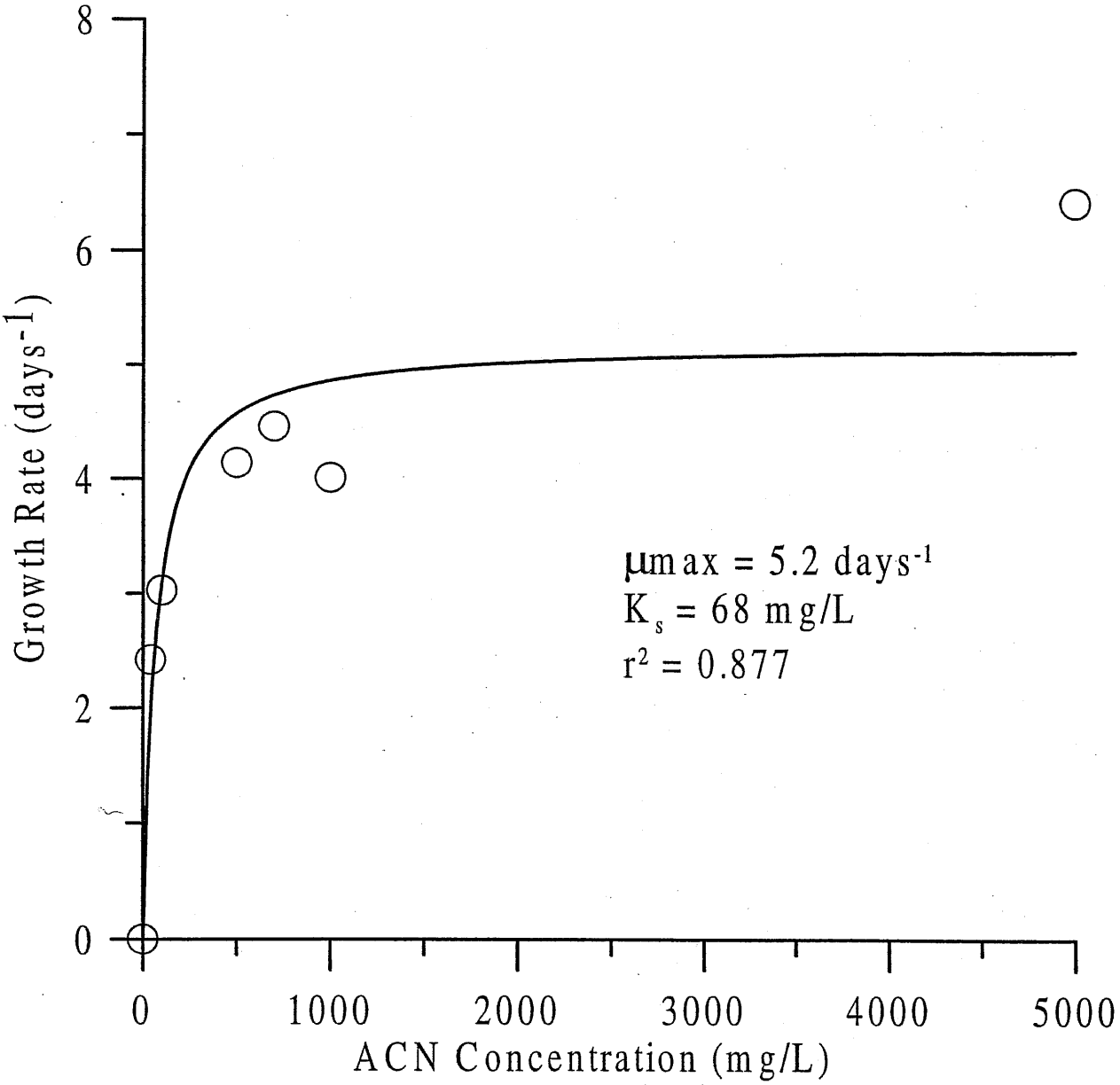

Figure 3: Growth of bacteria on methanol. Bacterial growth on methanol can be described using the Monod model

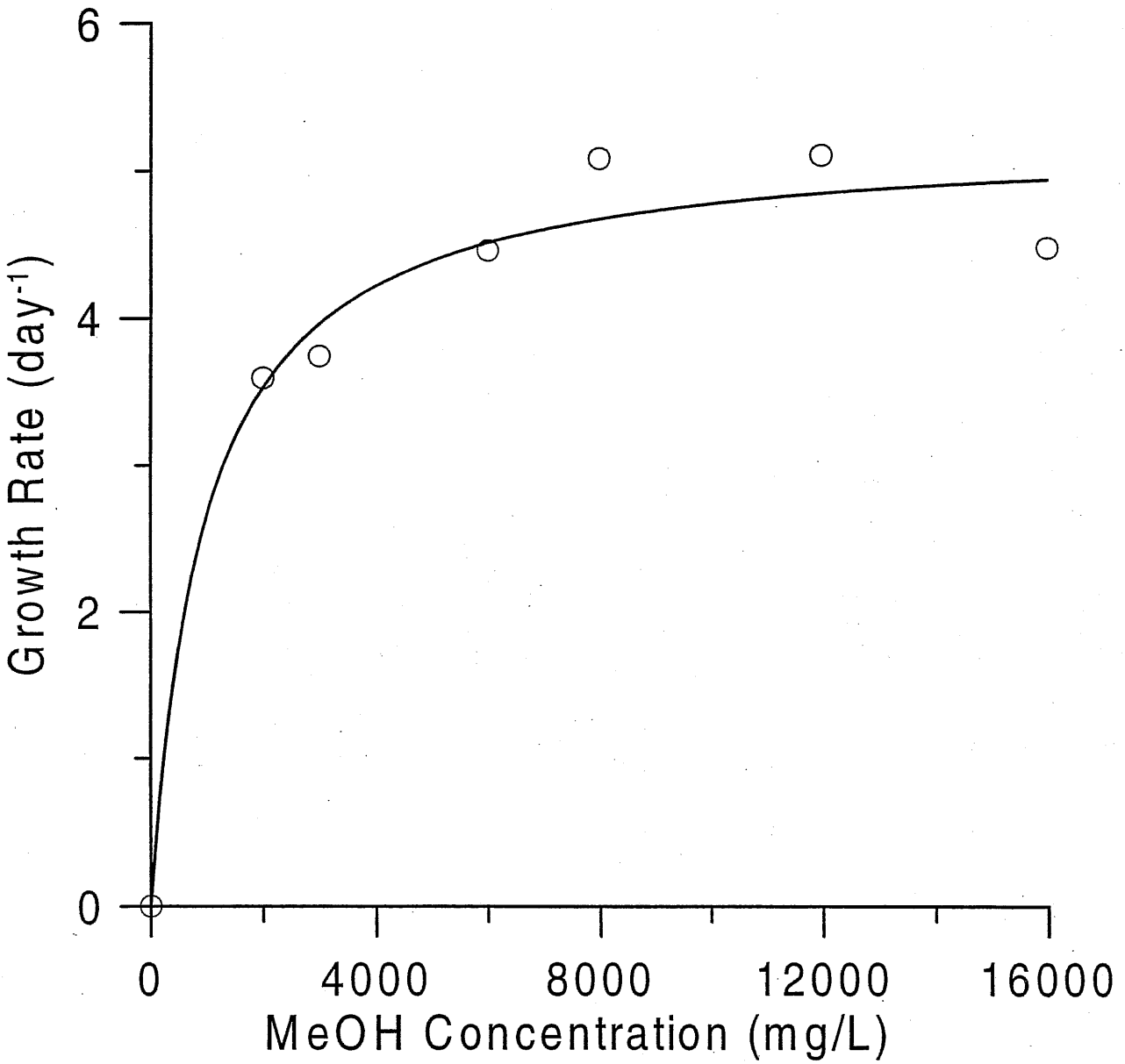

Figure 4: Bacterial oxidation of acetonitrile as a function of concentration.

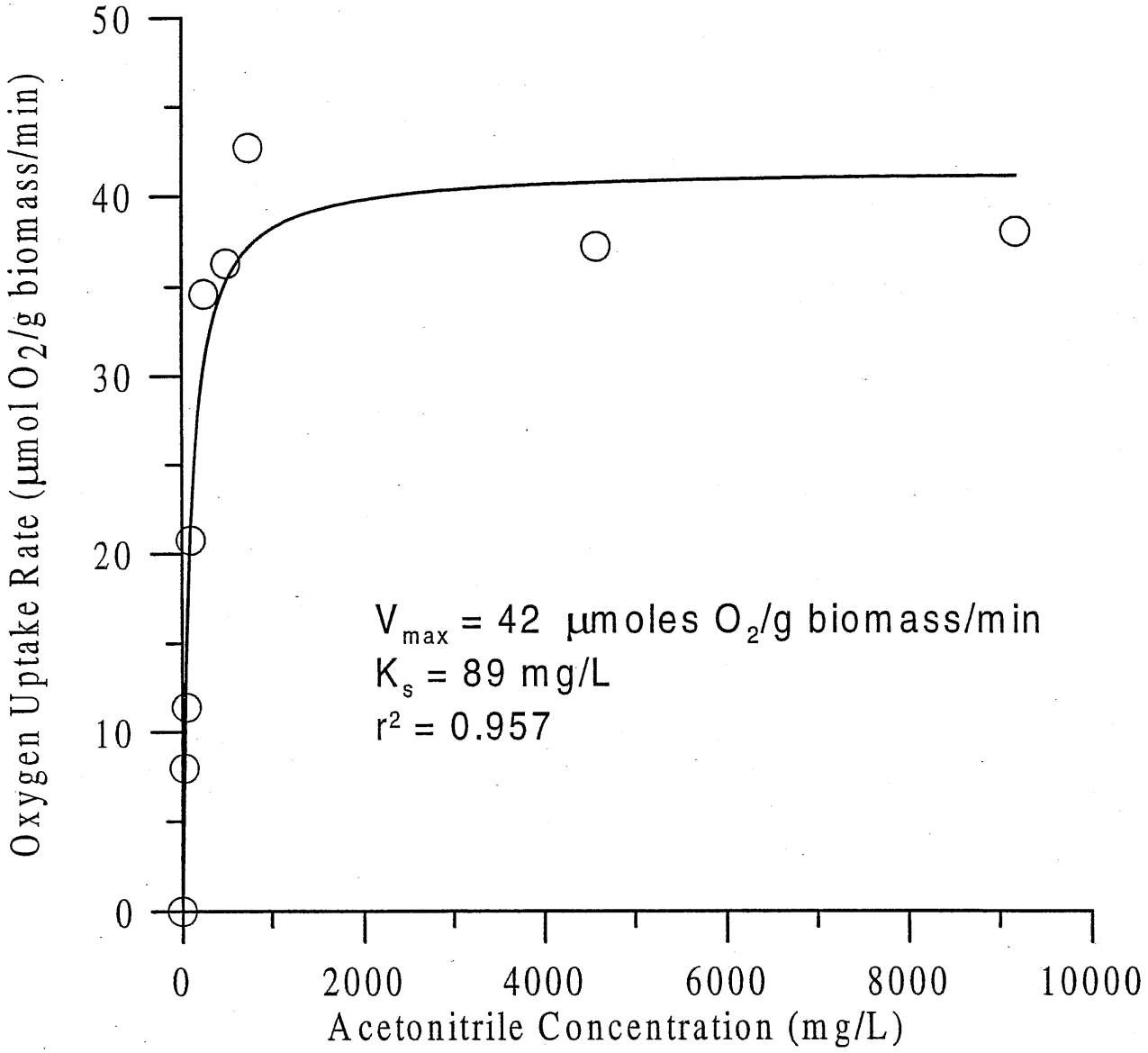

Figure 5: Bacterial oxidation of methanol as a function of concentration.

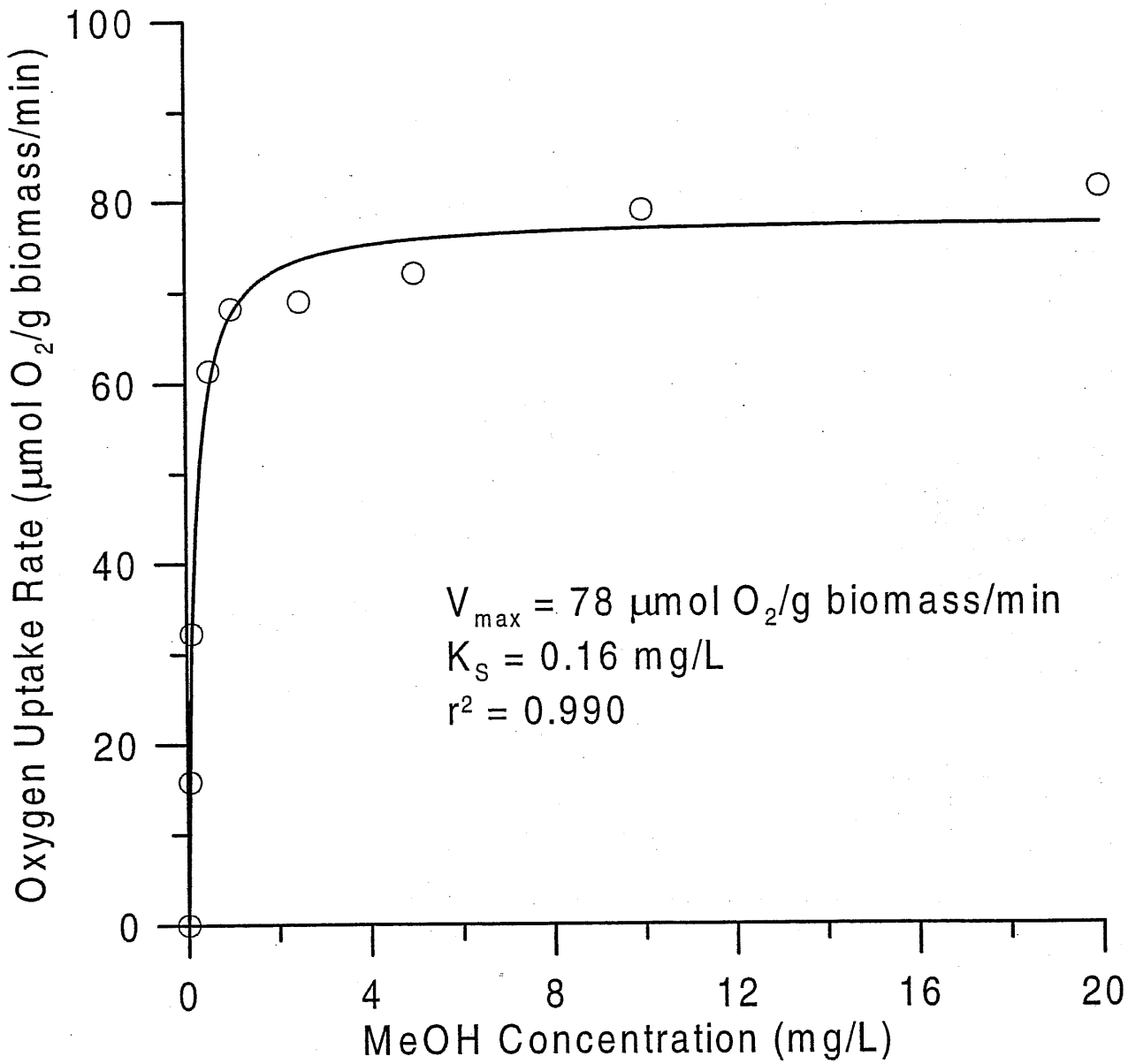

Figure 6. Effect of TFA on oxygen uptake rate of acetonitrile-enriched bacteria culture